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The isoporous substructure of the human glomerular slit diaphragm

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The isoporous substructure of the glomerular slit diaphragm in man. The substructure of the human glomerular slit diaphragm is examined after perfusion fixation of a human kidney with tannic acid-glutaraldehyde. The present study shows that the substructure of the slit diaphragm in man is very similar to that described in rats and mice, and that the dimensions of the contained pores (50 × 120 Å) in the human differ only slightly from those reported for the murine kidney. It is unlikely that rectangular pores of the dimensions of those measured in the human kidney would permit, under physiological conditions, the penetration of significant quantities of serum protein molecules. It is possible, therefore, that the slit diaphragm in the human glomerulus could function as a fine filter for serum proteins, although this remains to be definitely proved.

Ultrastructure du "slit diaphragm" glomérulaire chez l'homme. L'ultrastructure du "slit diaphragm" du glomérule humain a été étudiée après fixation par perfusion du rein au moyen d'acide tannique et de glutaraldéhyde. Ce travail montre que l'ultrastructure du "slit diaphragm" de l'homme est très semblable à celle décrite chez le rat et la souris et que les dimensions des pores qu'il contient (50 × 120 Å) ne sont que peu différentes de celles qui ont été obtenues dans les reins murins. Il est peu probable que des pores rectangulaires ayant les dimensions mesurées dans le rein humain permettent, dans des conditions physiologiques, la pénétration de quantités significatives de molécules de protéines sériques. Il est donc possible que le "slit diaphragm" du rein humain puisse fonctionner comme un filtre fin pour les protéines sériques encore que cela reste à démontrer.

The glomerular filtering capillary wall is a complex structure comprised of at least three layers: the fenestrated endothelium, the three-layered glomerular basement membrane (GBM) and the slit diaphragm which spans the space between epithelial foot processes. Although physiological studies [1] indicate that the glomerular filter permits the passage of molecules whose hydrodynamic radius is no greater than 36 Å, the precise location of the filtration barrier or barriers has not been unequivocally established. Ultrastructural studies, in which electron-dense tracers were used, have variously indicated that the barrier resides solely in the GBM [2, 3] or that the GBM is a coarse filter which acts in series with a fine filter localized in the slit diaphragm [4].

A recent study, in which a newly developed fixative was used, has shown that in mice and rats, the slit diaphragm has a highly ordered substructure [5]. When viewed *en face*, the diaphragm contains a central filament measuring 110 Å in width which runs parallel to and equidistant from the adjacent foot processes. On either side of the central filament, alternating cross-bridges span the space between the central filament and the neighboring foot processes, thereby defining a uniform population of rectangular pores measuring 140 × 40 Å [5]. The correspondence between these dimensions and those calculated from physiological data [1] has led to the suggestion that the slit diaphragm may function as the "small pore" system, or fine filter of the renal glomerulus [5]. The purpose of the present study was to establish whether a similar structure is present in the human glomerulus, and to determine whether its pore dimensions resemble those of murine kidneys.

Methods

Renal tissue was obtained from a child undergoing nephrectomy for Wilms's tumor. The patient was a 6½-yr-old white girl who had been in excellent health until May, 1974, when a large mass was palpated in her left upper quadrant. At the time of surgery the child had neither hypertension nor proteinuria, and on

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June 22, 1974 the left kidney was removed. The specimen weighed 960 g and showed a large tumor arising in the superior portion, which partially compressed renal veins and the adjacent renal tissue. Microscopically the tumor was a typical Wilms's tumor. The renal tissue that was not replaced by tumor appeared normal grossly and microscopically.

Immediately following excision of the kidney, the left renal artery was cannulated and 500 ml of Ringer's lactate (McGaw, Inc., Glendale, CA) containing 5000 U.S.P. units of sodium heparin (Organone, Inc., W. Orange, NJ) was infused at a hydrostatic pressure of 150 cm H_2O. The effluent drained freely from the left renal vein. The kidney was then fixed by perfusion with a solution of tannic acid-glutaraldehyde (TAG) [5] consisting of 1.5% tannic acid (Fisher Scientific Co., Pittsburgh, PA) and 1% glutaraldehyde in 0.1M phosphate buffer, pH 7.4. Perfusion fixation was continued for 15 min, at which time 1- to 2-mm thick slices of renal tissue were removed and immersed in the same fixative for an additional two hours at room temperature. After two 15-min rinses at 4°C in 0.1M sucrose, the tissue was postfixed in 2% aqueous OsO_4 for 1½ hr at 4°C. Staining *en bloc* with 1.5% uranyl acetate in 0.05M maleate buffer, pH 6.2, was carried out for one hour at 4°C. The tissue was then dehydrated in ethanol and embedded in epoxy resin (Epon). Silver or gray thin sections were cut with a diamond knife on an LKB Ultrotome III (LKB Instruments, Inc., Bromma, Sweden), picked up on carbon-coated grids and stained with lead citrate. They were examined in an electron microscope (Philips 300) operated at 60 kv, equipped with an anticontamination device and a 30 μ objective aperture. Microscope magnifications were calibrated with a diffraction grating replica 2160 lines/mm (Ladd Industries, Burlington, VT). Dimensions of the pores were measured directly from negatives using an LP-6 profile projector and micrometer stage (Ehrenreich Photo-Optical Industries, Garden City, NJ).

Results

Following TAG fixation, the human glomerulus showed certain ultrastructural features that differed

Fig. 1. *Human glomerular capillary wall from kidney fixed by perfusion with tannic acid-glutaraldehyde* (× *168,000*). The slit pore membrane (*arrows*) consists of a single electron-dense line in the middle of which is a small, black dot measuring 105Å in diameter. Irregular collections of electron-dense granules present in the lamina rara externa (*arrow heads*) may represent serum proteins which were incompletely removed by the preliminary washout procedure.

from those seen after conventional fixation; notably, extracellular structures including the GBM, mesangial matrix and glycocalyx were more electron-dense (Fig. 1). The three layers of the GBM were distinctly demarcated with the central lamina densa forming a dense meshwork of irregular fibrils ranging from 35 to 110 Å in thickness. The loosely meshed lamina rara interna and the lamina rara externa contained irregular particles of electron-dense material. In the lamina rara externa, some of these particles formed variously sized aggregates up to 600 Å in diameter. Because partial occlusion of the renal vein by tumor precluded the complete flushing of the renal vascular system of blood, these were interpreted as possibly being residual collections of serum proteins. From the base of each foot process irregular fibrils, measuring 35 to 100 Å in thickness, radiated toward the lamina densa. A small amount of amorphous electron-dense material was present in the space between foot processes immediately below the slit diaphragm.

Cellular membranes in general, and the plasma membranes in particular, had a distinct trilaminar appearance. In contrast to conventionally fixed kidneys, the microfilaments and microtubules of glomerular cells were clearly visible. The entire glomerular epithelial cell surface facing the urinary space was covered by a fuzzy coat of glycocalyx which, however, did not extend over the slit diaphragm. Unlike the podocytes of kidneys fixed in partially reduced osmium tetroxide [6], the foot processes of TAG-fixed kidneys were well separated one from the other, and there was no contact made between the glycocalyx layer of adjacent podocytes. The overall geometry of the glomerulus, however, was no different from that observed after perfusion fixation with aldehyde fixatives.

Glomerular slit diaphragm. In thin sections cut perpendicular to the plane of the GBM, the slit diaphragm was a single black line measuring between 60 and 75 Å in thickness (Fig. 1). Very frequently a central dot measuring 105 Å in diameter was seen in the diaphragm. The width of the slit diaphragm, as measured from the outer leaflet of the unit membrane of one foot process to the next, was between 320 and 395 Å with a mean of 364 Å. There was an increased density of the cytoplasm adjacent to the site of attachment of the slit diaphragm to the foot process membrane. As in the murine kidney, the site of attachment of the slit diaphragm on the side of the foot processes was

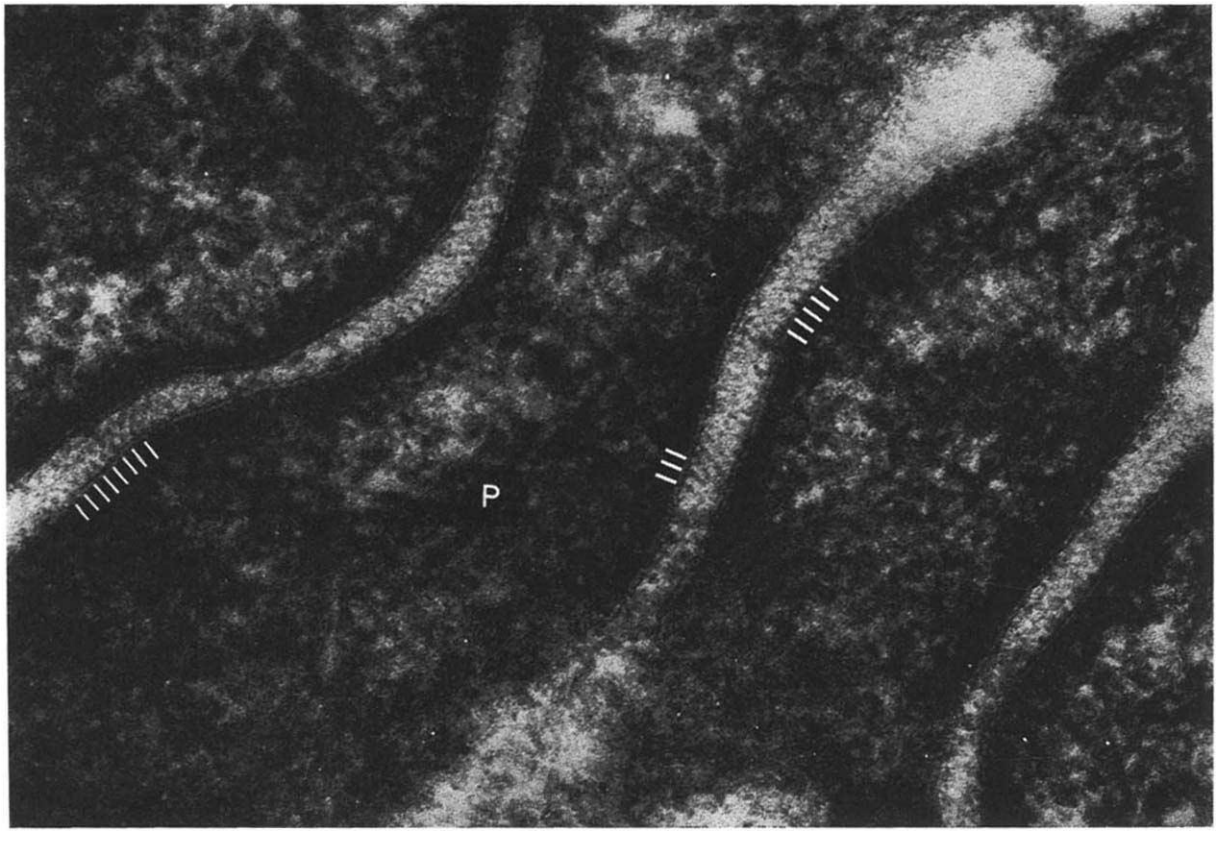

Fig. 2. *En face view of slit pore membranes present between epithelial foot processes (P) (× 175,000).* Areas in which the cross-bars are clearly delineated are indicated by a series of *short white lines.* In these areas the central filament is also more clearly visible.

approximately 500 to 700 Å above the plane of the outer surface of the GBM.

Tangential sections cut in the plane of the slit diaphragm showed that the latter had a highly ordered substructure (Fig. 2). An irregular central filament running along the long axis of the diaphragm and measuring between 110 and 120 Å in width was present equidistant from the unit membranes of adjacent foot processes. The central filament was connected to the adjacent foot process membranes by regularly spaced cross-bars measuring 70 ± 2 Å in thickness, 120 ± 2 Å in length and having a center to center spacing of 122 ± 2 Å. These regularly spaced cross-bars enclosed rectangular, electron-lucent spaces, the dimensions of which were calculated from the above measurements to be 50 × 120 Å. Due to the fact that the positions of the cross-bars on one side of the central filament alternated with those on the opposite side, the slit diaphragm had a zipper-like appearance similar to that described in the murine kidney.

In order to further compare the dimensions of the pores in the human slit diaphragm with those reported for the mouse and rat, the fractional pore area was calculated. As described by Rodewald and Karnovsky [5], the area occupied by the pores in the slit diaphragm was estimated as a percentage of the total surface area of the capillary wall. To do this, lines were drawn on electron micrographs showing tangentially sectioned slit diaphragms and foot processes, and the segments of the lines falling on the slit diaphragms were added and found to be 10% of the total length. From the dimensions indicated in Table 1, it was determined that the rectangular pores occupied 27% of the surface area of the slit diaphragm. From the latter two percentages it was calculated that the pores must occupy 2.7% of the glomerular capillary surface area, a value which is similar to that calculated for the murine kidney. As indicated by Rodewald and Karnovsky [5], it is probable that this value is an overestimation since the axillary portions of the capillary wall are occupied by the cell bodies of endothelial cells and lack slit diaphragms. In the human kidney, therefore, the surface area occupied by the pores themselves is estimated to be closer to 1.5 to 2% of the capillary surface area.

Table 1. Dimensions of slit diaphragm in man as compared to mouse and rat

	Human Å	Rat[a] Å	Mouse[a] Å
Width of whole diaphragm (25)[b]	364 ± 6[c]	394 ± 4	380 ± 5
Diameter of central filament (21)	116 ± 6	109 ± 2	110 ± 2
Diameter of cross bars (40)	70 ± 2	70 ± 2	67 ± 2
Center to center spacing of cross bars (45)	122 ± 2	112 ± 1	110 ± 1

[a] Data obtained from [5].
[b] Numbers in parentheses indicate the number of measurements made.
[c] Mean ± SEM.

Discussion

The present study shows that after TAG fixation, the substructure of the human slit diaphragm is clearly similar in appearance to that described in rats and mice [5]. The presence of this highly ordered isoporous substructure in all species thus far examined (mouse, rat and man), as well as its apparent barrier function in protein tracer studies [13], suggests that the slit pore diaphragm may play a role in glomerular ultrafiltration of macromolecules. This is further supported by the fact that the dimensions of the individual rectangular pores in the human kidney are comparable to those described in the murine kidney. While the present measurements on the human slit pore diaphragm must be viewed as an estimate, primarily because the conditions of perfusion fixation were not as optimal as can be achieved in experimental animals, it is nonetheless encouraging that their dimensions (50 × 120 Å) are such that with normal glomerular filtration, they would probably not permit the passage of significant quantities of serum albumin. The dimensions of the latter have been measured by a number of physical-chemical means. These indicate that the albumin molecule appears to be a prolate ellipsoid measuring approximately 150 by 38 Å [7], 168 × 34 Å [8, 9] and 129 × 39 Å [10], and physiological experiments indicate that serum albumin behaves as though it were a hydrodynamic sphere with a radius of 36 Å [11]. A molecule of this size might be expected, under physiological conditions, to be restricted in its passage through pores measuring 50 × 120 Å.

A discussion of the possible function of the slit pore diaphragm is best carried out in light of what has been deduced from physiological and ultrastructural tracer experiments on the permeability of the renal glomerulus. On the basis of data obtained from the clearance of several types of proteins relative to creatinine, Landis and Pappenheimer suggested that the renal glomerulus functions as though it were a semipermeable, isoporous membrane containing water-filled pores measuring between 35 and 42 Å in radius [11]. With pores of these dimensions, protein molecules larger than albumin (a_e, 35.5 Å, 68,000 daltons) are virtually excluded from entering the urinary space.

Although the precise location of the filtration barrier(s) in the glomerulus remains to be unequivocally established [12], considerable direct evidence has been obtained from experiments using macromolecular tracers of varying molecular weight which can be visualized with the electron microscope [4]. Farquhar, Wissig and Palade showed that the passage of large protein molecules, such as ferritin (500,000 daltons), is severely restricted by the central lamina densa of the GBM [2]. The role of the GBM in glomerular filtration has recently been reexamined by Caulfield and Farquhar [3] in a study using monodisperse dextrans of varying molecular weight. The results were interpreted as showing that the GBM is the primary glomerular filtration barrier. By contrast, experiments with smaller protein tracers suggested that in addition to the GBM, the slit pore membrane also appears to have a filtering function [13, 14]. These observations led Karnovsky and Ainsworth to postulate that there are two sites in series within the glomerular capillary wall: *a*) the GBM, and in particular the central lamina densa, which acts as a coarse filter; and *b*) the slit pore diaphragm which acts as a fine filter [4].

In a recent detailed review, Renkin and Gilmore [1] estimated the dimensions of the membrane openings and their cumulative area per unit path length for three theoretical models of the glomerular filter: one having either cylindrical pores of 36 Å radius, or slits of 36 Å half-width, or one composed of a fibrous meshwork. The values thus obtained were compared with the measured hydraulic conductivity of the glomerular membrane. The best agreement was obtained with the cylindrical pore model, which led the authors to suggest that *a*) a single restrictive barrier probably constitutes the site of both hydraulic conductivity and molecular sieving, *b*) the molecular restriction by other layers of the glomerular membrane in series with the restrictive barrier must be relatively small in comparison and *c*) the restrictive barrier would be best represented by a diaphragm containing regular perforations. These conclusions are consistent with those of Hall [15], Landis and Pappenheimer [11] and Karnovsky and Ainsworth [4] who, on the basis of physiological and ultrastructural evidence, suggested that some element in the slit pore complex is the principal site of glomerular protein filtration. The presence of a regular, isoporous substructure in the slit diaphragm of mice, rats and man, as well as the dimensions of its contained pores, support the hypothesis that this structure is a filtration barrier of the glomerulus. However, its role in protein filtration, whether a direct or indirect one, remains to be established.

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References

1. Renkin EM, Gilmore JP: Glomerular filtration, in *Handbook of Physiology*, Section VIII, *Renal Physiology*, edited by Orloff J, Berliner RW, Baltimore, Maryland, Williams and Wilkins Co., 1974, vol. VIII, pp. 185–248
2. Farquhar MG, Wissig SL, Palade GE: Glomerular permeability: I. Ferritin transfer across the normal glomerular capillary wall. *J Exp Med* 113:47–66, 1961
3. Caulfield JP, Farquhar MG: The permeability of glomerular capillaries to graded dextrans: Identification of the basement membrane as the primary filtration barrier. *J Cell Biol* 63:883–903, 1974
4. Karnovsky MJ, Ainsworth SK: The structural basis of glomerular filtration, in *Advances in Nephrology from the Necker Hospital*, edited by Hamburger J, Crosnier J, Maxwell MH, Chicago, Year Book Publishers Inc., 1972, pp. 35–60
5. Rodewald R, Karnovsky MJ: Porous substructure of the glomerular slit diaphragm in the rat and mouse. *J Cell Biol* 63:423–433, 1974
6. Schneeberger EE, Leber PD, Karnovsky MJ, McCluskey RT: Altered functional properties of the renal glomerulus in autologous immune complex nephritis. *J Exp Med* 139: 1283–1302, 1974
7. Oncley JL, Satchard G, Brown A: Physical-chemical characteristics of certain of the proteins of normal human plasma. *J Phys Colloid Chem* 51:184–198, 1947
8. Baldwin RL, Gosting LJ, Williams JW, Alberty RA: Transport processes and the heterogeneity of proteins. *Discuss Faraday Soc* 20:13–24, 1955
9. Wagner ML, Scheraga HA: Gouy diffusion studies of bovine serum albumin. *J Phys Chem* 60:1066–1076, 1956
10. Tanford C: *Physical Chemistry of Macromolecules*. New York, John Wiley and Sons, Inc., 1961
11. Landis EM, Pappenheimer JR: Exchange of substances through the capillary walls, in *Handbook of Physiology*, Section II, *Circulation*, edited by Hamilton WF, Dow P, Baltimore, Maryland, Williams and Wilkins Co., 1963, vol. II, pp. 961–1034
12. Schneeberger EE: Glomerular permeability to protein molecules: Its possible structural basis. *Nephron* 13:7–21, 1974
13. Venkatachalam MA, Karnovsky MJ, Fahimi HD, Cotran RS: An ultrastructural study of glomerular permeability using catalase and peroxidase as tracer molecules. *J Exp Med* 132:1153–1167, 1970
14. Graham RC, Karnovsky MJ: Glomerular permeability: Ultrastructural cytochemical studies using peroxidases as protein tracers. *J Exp Med* 124:1123–1133, 1966
15. Hall V: The protoplasmic basis of glomerular ultrafiltration. *Am Heart J* 54:1–9, 1957